

## Sporulation

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### How do *Bacillus subtilis* spores resist to ultra-high vacuum?

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Spore forming bacteria, such as *Bacillus subtilis*, are considered of great importance in space-life sciences, mainly because of the spores' multi-resistant characteristics. This sets spores as potential contaminants, posing a threat to food preservation as well as to planetary protection. It is the spores' overall structure that enables them to be so resilient, and efforts to study their resistance mechanisms to several space conditions have been undertaken. However, the effects of ultra-high vacuum in spores' viability are not well-known. Thus, it is our interest to open the spores' molecular treasure box and unravel the molecular mechanisms of spores' resistance to desiccation conditions in ultra-high vacuum.

For that, air-dried spores of *B. subtilis*, representing a wide set of 28 strains, were immobilized on stainless steel discs and exposed to ultra-high vacuum for 7 days. Each strain counts with specific knockout genes associated with numerous known molecular mechanisms of spore resistance to different space conditions: SASP (*sspA*, *sspB*, *sspE*), DPA (*spoVF*), core water content (*dacB*), spore coat layer (*cotE*, *safA*, *cotE safA*), spore crust (*cotX cotYZ*, *cotVW*), DNA repair by HR (homologous recombination), NHEJ (non-homologous end-joining), BER (base excision repair) (*recA*, *ku ligD*, *nfo*, *exoA*) and detoxification (*mrgA*, *katX*).

Spore resistance to ultra-high vacuum showed to be mainly dependent on SASPs, which are crucial in DNA protection. Moreover, DNA repair in the exposed spores showed to involve mostly ExoA Nfo, meaning BER plays a more important role than HR (RecA), NHEJ and detoxification (KatX).